Detection of MRSA nasal colonisation using the BD GeneOhm™ MRSA Assay

The BD GeneOhm™ MRSA Assay is a qualitative in vitro test for the rapid direct detection of methicillin-resistant S. aureus (MRSA) nasal colonisation. The test performed on the Smart Cycler® utilises polymerase chain reaction (PCR) for the amplification of MRSA DNA and fluorogenic target-specific hybridisation probes for the detection of the amplified DNA. The procedure takes about 60-75 minutes, depending on the number of specimens processed, allowing same-day reporting within a few hours of specimen collection.

Several studies have demonstrated up to 70% of hospitalised patients colonised with MRSA may only be detected by performing active surveillance cultures on muco-cutaneous colonisation sites including the anterior-nares and throat. Subsequently, MRSA surveillance cultures have become part of the clinical practice recommendations in both Europe and the USA. Several overseas studies have recently compared the BD GeneOhm MRSA Assay with culture against a panel of 10 hospital-associated and 50 community-MRSA (CA-MRSA) clones previously isolated in Western Australia (WA). The 60 MRSA clones were from multiple molecular backgrounds. The assay detected 57 of the 60 strains, which account for approximately 99% of MRSA isolated in WA. The full results of this study have been submitted for publication. In a similar study by Francois et al., 6% of strains were also found to have false negative results by the BD GeneOhm MRSA Assay, including two SCCmec type IV isolates, one SCCmec type III isolate and three SCCmec type V isolates.

Although culture-based methods are used by most laboratories for the detection of MRSA colonisation, recent mathematical modelling studies have suggested direct speciem MRSA detection on hospitalised patients by rapid and accurate molecular-based methods may help prevent MRSA transmission and be cost-beneficial in both the endemic and low prevalence setting.

Several potential benefits of using molecular methods for the rapid identification of MRSA colonised patients have been described, including:

- Lower medical liability costs.
- Lower risk of glycopeptide-resistant enterococci and staphylococci emerging.

**Principle**

The BD GeneOhm MRSA Assay is a rapid real-time PCR method used for the direct detection of MRSA. The sequences targeted in the assay are within the right extremity of the staphylococcal cassette chromosome mec (SCCmec), the mobile genetic element which harbours the mecA gene, and the orfX, a highly conserved open reading frame in S. aureus which is the site of SCCmec integration into the genome. Five primers targeting the SCCmec right-junction sequences corresponding to SCCmec types I, II, III, IVa, IVb and IVc are combined with one primer and three molecular beacons specific for the orfX gene. The amplified DNA targets are detected with molecular beacons, hairpin-forming single-stranded oligonucleotides labelled at one end with a quencher and at the other end with a fluorogenic reporter dye (fluorophore). In the absence of target, the fluorescence is quenched. In the presence of target, the hairpin structure opens upon beacon/target hybridisation, resulting in emission of fluorescence. The assay includes an internal control to detect PCR inhibitory specimens and to confirm the integrity of assay reagents.

**Performance**

The Gram-positive Bacteria Typing and Research Unit (GPBTRU) recently evaluated the BD GeneOhm MRSA Assay against a panel of 10 hospital-associated and 50 community-MRSA (CA-MRSA) clones previously isolated in Western Australia (WA). The 60 MRSA clones were from multiple molecular backgrounds. The assay detected 57 of the 60 strains, which account for approximately 99% of MRSA isolated in WA. The full results of this study have been submitted for publication. In a similar study by Francois et al., 6% of strains were also found to have false negative results by the BD GeneOhm MRSA Assay, including two SCCmec type IV isolates, one SCCmec type III isolate and three SCCmec type V isolates.

Several overseas studies have recently compared the BD GeneOhm MRSA Assay with culture for the direct detection of MRSA in nasal swabs (Table 1). In these studies, the prevalence of S. aureus identified as MRSA ranged from 6-27%. Compared with culture, the sensitivity, specificity and positive and negative predictive values of the BD GeneOhm MRSA Assay ranged from 89-100%, 92-99%, 67-96% and 97-100%.

In a recent Australian study by van Hal et al., the BD GeneOhm MRSA Assay was evaluated with the GenoType MRSA Direct Assay and three selective MRSA agars on 205 samples from consecutive known MRSA-infected and/or colonised patients. Detection of MRSA by the BD GeneOhm MRSA Assay was the most sensitive method. The sensitivity, specificity and positive and negative predictive values for nasal swabs was 94%.
Conclusion

Although most studies have demonstrated the BD GeneOhm MRSA Assay when compared to culture has a high negative predictive value, genetic mutations in the right hand end of the SCCmec element may produce a false negative result. Consequently, when using the BD GeneOhm MRSA Assay, local epidemiology of MRSA clones should be monitored to minimise the risk of not detecting emerging MRSA clones.

In addition, many of these studies demonstrated that the assay has a low positive predictive value. This may be due to the presence of S. aureus with an inactivated meca gene or with genetic elements similar in structure to the SCCmec but encoding functions unrelated to methicillin resistance. A BD GeneOhm MRSA Assay positive result should therefore be confirmed by culture. A negative culture result, however, may be due to the presence of non-viable organisms or when the number of organisms in the specimen is below the analytical sensitivity of the test. Consequently, a positive BD GeneOhm MRSA Assay result should not be used to guide or monitor treatment of MRSA but used as an adjunct to nosocomial infection control efforts to identify patients requiring enhanced precautions.

To maximise the full potential of a rapid molecular based method for the direct detection of MRSA nasal colonisation, both the laboratory and hospital’s infection control work practices require re-evaluation and possible modification.

References


Table 1. Comparison of the BD GeneOhm MRSA Assay to culture for the detection of MRSA in nasal swabs.

<table>
<thead>
<tr>
<th>Study Authors</th>
<th>No.</th>
<th>Culture positive</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warren et al.</td>
<td>288*</td>
<td>80</td>
<td>92</td>
<td>94</td>
<td>82</td>
<td>97</td>
</tr>
<tr>
<td>Oberdorfer et al.</td>
<td>304*</td>
<td>16</td>
<td>100</td>
<td>99</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Bishop et al.</td>
<td>192*</td>
<td>26</td>
<td>89</td>
<td>95</td>
<td>72</td>
<td>98</td>
</tr>
<tr>
<td>de San et al.</td>
<td>522*</td>
<td>32</td>
<td>91</td>
<td>97</td>
<td>67</td>
<td>99</td>
</tr>
<tr>
<td>Paule S et al.</td>
<td>403*</td>
<td>50</td>
<td>98</td>
<td>96</td>
<td>77</td>
<td>100</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>398</td>
<td>27</td>
<td>100</td>
<td>97</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td>Boyce J et al.</td>
<td>286</td>
<td>63</td>
<td>100</td>
<td>99</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Farley et al.</td>
<td>599</td>
<td>91</td>
<td>89</td>
<td>92</td>
<td>66</td>
<td>98</td>
</tr>
</tbody>
</table>

* Enrichment broth used